

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,500

Open access books available

136,000

International authors and editors

170M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Insights into the Role of Defective Apoptosis in Cancer Pathogenesis and Therapy

*Sonia Thapa, Rafiq A. Rather, Shashank K. Singh  
and Madhulika Bhagat*

## Abstract

One form of programmed cell death (PCD) is apoptosis. Defective apoptosis is an indispensable causative factor in the development of cancer that allows cancer cells to survive longer and favors the accumulation of oncogenic mutations. Further, upregulation of antiapoptotic proteins (e.g., Bcl-2, Mcl-1) and loss of pro-apoptotic proteins (e.g., Bid, Bad, Bax, Bak) strongly favors apoptosis evasion. The ability of cancer cells to evade apoptosis is critical for the progression and clonal expansion of malignantly transformed cells. Defective apoptosis imparts proliferative advantage to cancer cells or cells with the potential to become cancerous. The mechanisms employed by cancer cells to evade apoptosis can be used in the strategic design of therapeutic regimens aimed at exploiting apoptotic signaling networks to ensure tumor-specific cell death. Therefore, to ensure tumor-specific cell death, we may need to exploit the expression and/or function of different components of apoptotic signaling that are critical for maintaining cell survival and are regulated differently in tumor cells than normal cells. Both inhibitors of anti-apoptotic proteins and activators of pro-apoptotic proteins can be used for cancer therapy. In this chapter, we attempted to summarize the knowledge about the molecular mechanisms of defective apoptosis that could be translated into the development of novel therapeutic agents and therapeutic modalities for cancer treatment.

**Keywords:** apoptosis, cancer, cell signaling, cancer therapeutics, drugs

## 1. Introduction

The term apoptosis was coined by Kerr, Wyllie and Currie in 1972 to describe a form of programmed cell death. This type of cell death is mediated by intracellular proteolytic enzyme cascades in a highly regulated fashion. The cells that die as a result of apoptosis typically do not burst and do not release their intracellular contents in the surroundings [1]. As a result apoptosis is typically not associated with inflammation. In multicellular organisms, this process participates in development, tissue homeostasis, and acts as a defense mechanism against the formation of genetically altered cells. Apoptosis is a pivotal for maintaining normal cell turnover and offers defense strategy against tumorigenesis in multicellular organisms. Consequently cells with unrepairable genetic damage or the cells with potential to become cancerous are eliminated via apoptosis. Thus aberrant failure

or deficiency of apoptotic signaling can lead to unregulated growth of genetically altered cells and subsequently the development of cancer [2]. Similarly, over activation of apoptosis may result in excessive death of normal cells and may lead to development of neurodegenerative disorders such as autoimmune disorders, Parkinson's disease, Huntington's disease, and Alzheimer's disease [3]. Both failure of apoptosis and excess of apoptosis are detrimental for an organism. Apoptosis is highly programmed biological process that occurs during both physiological and pathological states. An in-depth knowledge of apoptosis is critical for understanding the pathogenesis of many diseases [4]. For example, cancer is a disease condition where little or no apoptosis occurs resulting in unregulated growth of genetically altered cells [5]. Therefore, there is need to identify new drugs and new chemical entities that can potentially target various aspects of apoptosis in tumor cells selectively and specifically. In this chapter, we made an attempt to summarize the knowledge on apoptosis, its molecular mechanism and how defective or unregulated apoptosis leads to cancer development and how apoptosis can be used for therapeutic intervention of cancer. The process of apoptosis manifest multitude of morphological and biochemical changes which can be detected by various cell biological techniques.

### **1.1 Morphological characteristics of apoptosis**

During apoptosis, a cell undergoes a series of morphological changes such as condensation of cytoplasm, condensation of chromatin, nuclear fragmentation, cell rounding, cell shrinkage and blebbing of nuclear and cytoplasmic membranes to form membrane-bound fragments. The chromatin condensation typically begins at the ends of nuclear envelop that forms a ring-like structure [6]. The chromatin condensation continues until it disintegrates into small membrane-bound apoptotic bodies. These small bodies are crowded with closely packed cellular organelles and smaller fragments of nucleus [6, 7]. These apoptotic bodies are immediately taken up by phagocytes such as macrophages, dendritic cells and Langerhans cells. The engulfment of these apoptotic bodies by phagocytes occurs without any inflammation and release of intracellular contents. As we know, apoptosis is usually considered a non-inflammatory process while necrosis (another mode of cell death) activates inflammation. This apoptotic process is typically mediated by the proteolytic cleavage of cellular substrates by caspases, and signaling elements [7, 8]. The morphological changes occur in parallel with the activation of a number of complex biochemical effector pathways that cause solubilization of the apoptotic cells.

### **1.2 Biochemical characteristics of apoptosis**

Besides morphological changes, an apoptotic cell progresses through a series of biochemical changes such as endonucleolytic fragmentation of genomic DNA by endogenous DNases which cleave intranucleosomal regions of genomic DNA into double stranded DNA (dsDNA) fragments of sizes varying from 180 bp to 200 bp. DNA fragmentation is the frequent end point and the widespread marker of apoptosis [9]. The DNA fragments that are formed during apoptosis contain single base 3' overhangs as well as blunt ends. The DNA fragments formed during apoptosis are noticeable as a ladder pattern in the ethidium based-electrophoresis of genomic DNA [10]. The most important enzymes that catalyze the cleavage of genomic DNA during apoptosis include DNA fragmentation factor (DFF40), caspase activated DNase (CAD) and 70-kDa endonuclease (NUC70). In a normal healthy cell, DFF40 and CAD are retained as inactive heterodimers with inhibitor proteins DFF45 and ICAD (inhibitor of CAD). During apoptosis, these DNase are selectively activated

upon cleavage by caspase 3. Once activated, DFF40 and CAD are sufficient to induce the nuclear morphological changes characteristic of apoptosis. Similarly, when isolated HeLa nuclei are incubated with NUC70, the nuclei undergo inter-nucleosomal DNA fragmentation. NUC70 is a cytoplasmic endonuclease that is translocated to the nucleus after the initiation of apoptosis [7, 11].

## 2. Pathways of apoptosis

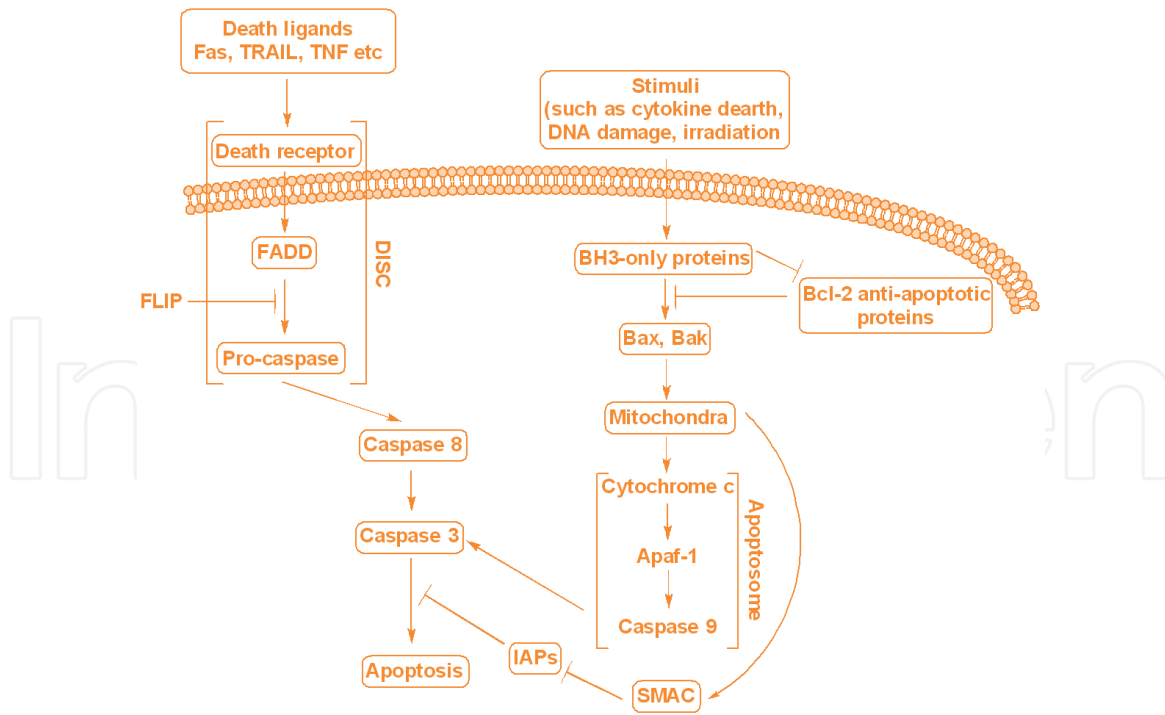
There are atleast two fundamental pathways of apoptosis: extrinsic, death receptor-mediated pathway and intrinsic, mitochondria-mediated pathway. Another important pathway of apoptosis is perforin/granzyme pathway. Apoptosis can be initiated through any one of these pathways.

### 2.1 The extrinsic death receptor pathway

This pathway begins when ligand binds to the death receptor, for example, with the attachment of extracellular ligands, like tumor necrosis factor (TNF), Fas ligand (Fas-L), TNF-related apoptosis-inducing ligand (TRAIL) to the extracellular domain of transmembrane receptors. There are six death receptors (TNFR1, Fas, DR3, DR4 [TRAILR1], DR5 [TRAILR2], and DR6) that have been identified in mammalian cells (**Table 1**). While signaling through TNFR1 and DR3 is proinflammatory in nature, signaling via other death receptors is predominantly pro-apoptotic. The death receptors bind via their intracellular death domain with adapter proteins such as Fas-associated death domain (FADD) and TNF receptor-associated death domain (TRADD). These adaptor proteins, called the Death effector Domain (DED) have another protein interaction domain [12]. These sequential steps leads to the formation of a death inducing signaling complex (DISC), that leads to auto-catalytic activation of procaspase-8 (**Figure 1**). Active caspase-8 activates effector/executioner caspases (*cysteine-dependent aspartyl-specific proteases*), which cause cell death by damaging the nucleus and other intracellular structures. Once caspase-8 is activated, the execution phase of apoptosis is triggered and activated. This type of apoptosis, which is death receptor-mediated apoptosis, can be inhibited by a protein called c-FLIP. c-FLIP bind to FADD and caspase-8, and turns them ineffective. A protein called Toso, has been shown to block Fas-induced apoptosis in T cells. This may be due to inhibition of caspase-8. The death receptors belong to the tumor necrosis factor (TNF) receptor gene superfamily. TNF family contains cysteine-rich extracellular domains and a cytoplasmic domain of about 80 amino acids called the “death domain”. This death domain plays a critical role in transmitting the death signal from the cell surface to the intracellular signaling pathways [13–15].

Death receptor	Activating ligand
TNFR1/DR1/CD120a/p55	TNF
Fas/CD95/Apo1/DR2	Fas/CD95/Apo1/DR2
DR3/Apo3/WSL-1/TRAMP/LARD	Apo3L/TWEAK
TRAIL-R1/DR4	TRAIL/Apo2L
DR6	TRADD

**Table 1.**  
*Death receptors and their cognate ligands.*



**Figure 1.**  
Key components of the intrinsic and extrinsic pathways of apoptosis.

## 2.2 The intrinsic mitochondrial apoptosis pathway

Intrinsic apoptosis is a form of regulated cell death that is activated in response to a variety of stimulus such as dearth of growth factors, DNA damage, endoplasmic reticulum (ER) stress, and overproduction of reactive oxygen species (ROS). The intrinsic pathway is affected by members of the Bcl-2 family proteins such as Bcl-2 and Bax. These proteins usually are bound to the outer mitochondrial membrane and act as antiapoptotic and pro-apoptotic proteins respectively [6]. In the intrinsic pathway the main consequence of proapoptotic signaling is mitochondrial membrane perturbation and release of cytochrome ‘c’ in the cytoplasm. Once released in the cytosol, cytochrome ‘c’ forms a complex with apoptotic protease activating factor 1 (APAF1) and inactive form of pro-caspase 9, commonly known as apoptosome (**Figure 1**). This complex hydrolyzes adenosine triphosphate (ATP) to cleave and activate caspase 9. The initiator caspase 9 then cleaves and activates the executioner caspase 3, caspase 6, and caspase 7, resulting in cell apoptosis. The antiapoptotic proteins Bcl-2 and Bcl-X<sub>L</sub> inhibit cytochrome ‘c’ release [8]. The Bcl-2 group of proteins share one to four Bcl2 homology (BH) domains (i.e., BH1, BH2, BH3, and BH4) [16]. Bax and Bak of the Bcl-2 family form pores across the outer mitochondrial membrane. Besides this, Bax also continuously cycles between the outer mitochondrial membrane (OMM) and the cytosol, and exhibits a quiescent inactive dimeric conformation [17]. Bak constitutively resides at the OMM, where it inserts within hydrophobic C-terminal of voltage dependent anion channel 2 (VDAC2). Upon apoptosis induction, mitochondrial pools of Bax and Bak undergo direct or indirect activation by pro-apoptotic proteins [16].

The BH3-only proteins are BCL2 binding component 3 or p53, Bcl-2-like 11 (BIM) [18]. Some internal stimuli cause an increase in mitochondrial membrane permeability and these stimulus send the signal to the mitochondria, that form mitochondrial outer membrane permeabilization (MOMP). MOMP is most commonly mediated via a variety of protein membrane and protein–protein interactions of the B-cell lymphoma 2 protein (Bcl-2) family [19–21].

Both extrinsic and intrinsic pathways terminate at the execution phase in the final stage of apoptosis. Activated execution caspases further activates cytoplasmic



endonucleases, proteases that degrade nuclear and cytoplasmic proteins respectively. The executioner caspases are caspase-3, caspase-6, and caspase-7 that cleave substrates like cytokeratins, PARP, and others that results in morphological and biochemical changes in apoptotic cells. The most important of the executioner caspases is caspase-3 and is activated by any of the initiator caspases (caspase-8, caspase-9, or caspase-10). Caspase-3 induces cytoskeleton reorganization and disintegration of the cell into apoptotic bodies [22].

### 2.3 Perforin/granzyme pathway

The granzymes belong to a family of serine proteases contained in cytotoxic granules of innate and adaptive immune killer cells. The key function of these enzymes is eliminating viruses and tumor cells. They also regulate immune cells and inflammation by controlling the survival of lymphocytes. Granzymes are expressed by three gene clusters. Granzyme A and granzyme B are the most abundant granzymes. Granzyme-mediated apoptosis is mainly used by lymphocytes to destroy virus infected cells. It is a kind of type IV hypersensitivity where sensitized CD8<sup>+</sup> cells kill antigen-bearing cells. The FasL/FasR interaction is the predominant method of CTL-induced apoptosis. They also involve pathways that include secretion of the transmembrane pore-forming molecule perforin with a subsequent release of cytoplasmic granules that have serine proteases as well as granzyme B, through the pore and into the target cell [23]. Granzyme B cleaves proteins at aspartate residues and can activate pro-caspase-10. It can also cleave factors like ICAD (Inhibitor of Caspase Activated DNase). Granzyme B can amplify the death signal by inducing cytochrome c release. It can also activate caspase-3 directly [24].

Therefore, in these types of pathways, signaling pathways that are upstream are bypassed and execution phase of apoptosis is directly influenced. It is suggested that both the mitochondrial pathway and direct activation of caspase-3 are critical for granzyme B induced killing activates both mitochondrial pathway and caspase-3 activation [24, 25]. The immune cells that express highly variable and regulated patterns of granzymes include natural killer (NK) cells, cytotoxic CD4 and CD8 T cells, and regulatory T cells (Tregs). The granules contain perforin, to deliver the granzymes into the target cell. When cytotoxic T lymphocytes (CTLs) and NK cells form an immune synapse with a target cell for its elimination, cytotoxic granules join immune synapse where its membrane fuses with the killer cell membrane. This action results in the release of the granule contents into the synaptic cleft. The granzymes are then initiate distinct pathways of programmed cell death [8, 26, 27].

### 3. Bio markers of apoptosis

A biomarker is measured and evaluated to indicate normal or diseased biological processes. It has the potential to enhance translational progress and accelerate drug development. They allow monitoring of drug efficacy and also help in preclinical drug evaluation. It also allows early detection of toxicity during drug evaluation. The fragmented DNA on agarose gel is a usual marker to detect apoptosis. The poly (ADP- ribose) polymerase (PARP) cleaved form, observed in cells undergoing apoptosis. Other markers are cytokeratin-18 cleavage by caspase 3. It may be detected by using the antibody M30. Cleavage of various caspases can be detected using flow cytometry during apoptosis [28, 29]. The widely used biomarkers of apoptosis, their methods of analysis and the specimen needed for analysis are discussed in **Table 2**. However, the use of these biomarkers as a tool to predict the occurrence of apoptosis in the pathogenesis of different diseases states warrants further investigation.

Biomarkers	Analysis method	Specimen for biomarker analysis
Cytochrome c	ELISA	Biomarker in blood samples
Activated caspases Caspase 2,3,7,8,9	ELISA Flow cytometry	Detection by substrate active site activation
Cytokeratins	ELISA Flow cytometry	Biomarker in blood samples
Bcl-2, Bcl-x <sub>L</sub>	ELISA Flow cytometry	Its over expression results in chemoresistance
Externalized Phosphatidylserine	ELISA Flow cytometry	Early apoptotic event. It measures annexin that binds to the external ligand
Fas, Fas ligand	ELISA Flow cytometry	Expression on B and T cells, also expressed on normal and tumor tissues.
Granzyme B	PET imaging	Response in immunotherapy
8-hydroxy-2-deoxyguanosine, 3-nitrotyrosine	ELISA	Biomarker in tissue or urine

**Table 2.**  
*Biomarkers of apoptosis.*

**4. Role of apoptosis in cancer**

Cells can die in variety of ways which includes apoptosis, necrosis, mitotic catastrophe, senescence, and autophagy. Of these different modes of cell death, apoptosis is active, programmed and genetically controlled. While physiological apoptosis helps to eliminate genetically altered cells, defective apoptosis is intimately connected with cancer pathogenesis [30]. Apoptotic signaling regulation is important to preserve a proper balance between cell death and cell survival. It is also important in maintaining genome integrity. The Apoptosis evasion is a prominent hallmark of cancer and cancer cells can use a number of diverse strategies to evade apoptosis. The disruption in the balance between pro- and anti-apoptotic proteins contributes to carcinogenesis [31]. It may be due to the reducing apoptosis in malignant cells [30]. For example, the imbalance between pro- and anti-apoptotic Bcl-2 proteins, its genetic and epigenetic alterations, can promote cancer cell survival. The elevated levels of anti-apoptotic family members is a distinct mechanism of apoptosis dysregulation in cancer. The anti-apoptotic proteins are widely over-expressed in cancer cells to overcome stress signals. Over-expression of anti-apoptotic Bcl-2 family proteins is often correlated with recurrence, poor prognosis, and resistance to cancer therapeutics [11].

miRNAs are a class of non-coding RNAs that regulate post-transcriptional gene expressions and silence target mRNAs. miRNAs dysregulation are associated with different human cancers and microRNAs (miRNAs) can function as oncogenes as well as tumor suppressors and their dysregulation are associated with many different human cancers.. miRNAs target different mRNAs and act as anti-apoptotic or pro-apoptotic regulatots that involved in the apoptotic pathways [32]. For example, miR-15/16 targets the anti apoptotic factor (Bcl-2). In many cancers, the mostly up-regulated miRNA is anti-apoptotic miRNA-21. It targets the programmed cell death 4 gene (PCD 4 gene or PDCD4), tropomyosin 1 (TPM1), and the phosphatase and tensin homolog (PTEN), to modulate apoptosis. The application of anti-microRNAs imitate may act as a potent therapeutic strategy to inhibit key molecular signaling pathways that are present in cancer [32, 33].

Cancer cells often show dysregulated expression patterns of diverse long non-coding RNAs (lncRNAs) in specific kinds of tumors. Therefore, their upexpression or down-regulation in cancer cells often sensitizes cells to apoptotic treatments.

They also induce and modulate apoptosis. Therefore, targeting lncRNAs in cancer cells can be utilized for cancer treatment. Cellular-FLICE inhibitory protein (c-FLIP) is a critical negative regulator of the apoptotic pathway. The apoptosis regulation due to c-FLIP, mediated by the death receptors Fas, TNF-R1, DR4 and DR5. It exists in three isoforms that are derived from diverse mRNA splice variants. These splice variants are transcribed under the same promoter, namely c-FLIPL, c-FLIPS, and c-FLIPR. The three isoforms of c-FLIP acts at the DISC level and inhibit the procaspase 8 and 10 activation. C-FLIP high expression is found in many cancers and its downexpression can restore apoptosis mediated by TRAIL A and CD95L. Thus, c-FLIP can act as a promising target for cancer therapy [34]. c-FLIP can also induce apoptosis at low and more physiologically relevant expression levels by recruiting at the DISC to increase caspase-8 activation.

NF- $\kappa$ B activation by CD40 ligand or TNF- $\alpha$  results in overexpression of c-FLIP and the prohibition of TNFR1, Fas- and TRAIL receptors induces apoptosis. Activation of several pathways, such as mitogen-activated protein kinase (MAPK), the phosphatidylinositol-3 kinase (PI3K)/Akt, can enhance the expression of c-FLIP and can hamper apoptosis induced by death receptors [35].

IAPs increase cell survival during cellular stresses such as ER stress and prevent both intrinsic and extrinsic apoptosis. Caspases dysregulation may inhibit apoptosis and carcinogenesis. Down-regulation of different caspases has been observed in many cancers. For example, caspase-9 and caspase-3 downregulation can lead to formation of different forms of cancers such as colorectal, ovarian, breast, and cervical cancers [36, 37].

## 5. Therapeutic targeting of defective apoptosis in cancer

Apoptosis plays a pivotal role in cancer pathogenesis. Therefore, understanding the molecular basis of defective apoptosis has garnered tremendous attention from medical researchers. Novel therapeutic agents and treatment modalities to modulate disease pathogenesis are under trials in both preclinical and clinical settings. An important strategy to modulate cancer pathogenesis involves the use of small molecule inhibitors (SMIs) that target specific components in apoptotic cascades.

### 5.1 Targeting anti-apoptotic Bcl-2 family members

Bcl-2 is over expressed in many types of tumors and imparts therapeutic intractability to such tumors. A highly selective inhibitor of Bcl-2 is Venetoclax. This drug has been approved for routine clinical practice and is currently in use for the treatment of ALL (acute lymphocytic leukemia), CLL (chronic lymphocytic leukemia), and multiple myeloma T-cell prolymphocytic leukemia. Venetoclax is a Bcl2-selective BH3-mimetic. BH3-mimetics represents a class of anticancer drug that mimic the functions of BH3-only proteins. These mimetics bind to prosurvival proteins like Bcl-2 and inhibit their ability to bind Bax or Bak [38]. Thus, when Bcl-2 overexpressing cancer cells are treated with venetoclax *in vitro*, the cancer cells undergo apoptosis. Venetoclax is often used alone or in combination with other drugs such as rituximab, ibrutinib, azacitidine/decitabine, and bortezomib/dexamethasone against various hematological malignancies. Bcl-2 family members over expression are affiliated with aggressive cancer and chemo resistant [39]. These credentials make these proteins as highly encouraging therapeutic targets to develop pharmacological anticancer drugs. Bcl-2 family members inhibition by small interfering RNAs (siRNAs) may also induce apoptosis and can reduce tumor growth [40]. For example, Mcl-1 down regulation by siRNA induced significant



apoptosis in leukemia cells. Many different microRNAs have been associated that regulate Bcl-2 expression such as miR-195, miR-24-2, and miR-365-2 act as negative regulators of Bcl-2 family, which shows the therapeutic potential of these miRNAs [16, 41].

## 5.2 Mcl-1 inhibitors

The anti-apoptotic protein myeloid cell leukemia-1 (Mcl-1) is an important regulator of apoptosis and a central driver of drug resistance in multitude of human malignancies. Overexpressed Mcl-1 imparts drug resistance of both solid tumors and hematological malignancies against various therapeutic agents. Several inhibitors have been developed that show promising anticancer activities in preclinical and clinical settings. For example S63845 induces apoptosis in SCLC cell lines in vitro at an IC<sub>50</sub> of 23 to 78 nM, while in xenograft models this molecule causes significant reduction in tumor volumes. S63845 can be used in combination with navitoclax (a dual inhibitor of Bcl-x<sub>L</sub> and Bcl-2) where S63845 reduced the cell viability of SCLC cells and showed synergistic effects in S63845-resistant xenograft models [42]. Furthermore, Mcl-1 is an attractive drug target in lung cancer due to its non-apoptotic involvement in DNA repair. Thus targeting Mcl-1 with a small molecule inhibitor (MI-233) blocks Mcl-1-mediated HR DNA repair and thereby sensitizes cancer cells to treatment induced replication stress. MI-233 shows strong synergism with hydroxyurea or olaparib in lung cancer models [43]. Similarly, targeted Mcl-1 inhibition by RNAi also increases caspase-mediated cell death in cell models such as ER $\alpha$  + breast cancer cells [44]. A specific Mcl-1 inhibitor VU661013 induce tumor cell death and a causes synergistic reduction when used in combination with ABT-263 in tumor volume [45]. During the last several years, many Mcl-1 inhibitors have been developed. However, due to large surface-exposed hydrophobic BH3 binding groove, specific targeting of Mcl-1 poses a big challenge. Thus indirect targeting of Mcl-1 is emerging as important mechanism of action of alternative drug classes such as CDK9 inhibitors or deubiquitination inhibitors [46]. The pharmacological characteristics of the major Mcl-1 inhibitors are tabulated in **Table 3**.

## 5.3 X-linked inhibitor of apoptosis protein (XIAP) inhibitors

Although IAPs control plethora of signaling pathways, they were initially thought to be responsible for caspase inhibition by acting as negative regulators of apoptosis [36, 37]. Many small molecules have been designed to target the IAP proteins and one such category of an molecules is Smac mimetics (SMs). A recently developed XIAP inhibitor BMT-062789 displays remarkable anticancer activity against a panel of lymphoma cell lines. BMT-062789 is a heterodimeric mimetic of the second mitochondrial activator of caspases (SMAC) [47]. This molecule inhibits both the caspase 9 and caspase 3/7 binding domains of XIAP [48]. When used in combination with etoposide, BMT-062789 induces apoptosis in rituximab resistant cell line models Raji 4RH and RL 4RH. ASTX660 is another orally bioavailable, non-peptidomimetic antagonist of both XIAP and cellular IAP1 (cIAP1), with potential antineoplastic and pro-apoptotic activities. ASTX660 selectively binds to and inhibits the activity of XIAP and cIAP1 [49].

## 5.4 Caspase activators in cancer therapy

Caspases constitutes a group of cytosolic aspartate-specific cysteine proteases that participate in the initiation and execution of apoptosis Pharmacological activation of caspases using small molecule activators is an effective therapeutic

Drug	Affinity (Ki)	Activity in tumors
S63845	Ki < 1.2 nM	non-small cell lung cancer, breast cancer, melanoma
AMG 176	Ki = 0.06 nM	breast cancer
AZD5991	Ki = 0.2 nM	multiple myeloma, acute myeloid leukemia
VU661013	Ki = 97 ± 30 pM	acute myeloid leukemia
Compound 42	Ki = 0.03 nM	triple-negative breast cancer, hematological malignancies
β-carboline copper(II) complexes	Ki = 1.2–96.4 nM	non-small cell lung cancer

**Table 3.**  
*Pharmacological characteristics of some Mcl-1 inhibitors.*

Caspase activator	Mechanism of action
Cisplatin	potent pro-apoptotic anticancer agent; activates caspase-3
α-(Trichloromethyl)-4-pyridineethanol (PETCM)	activator of caspase-3
Apoptosis activator 2	promotes apoptosome formation and activates caspase-9/caspase-3 pathway; selectively induces tumor cell apoptosis
4-(Phenylmethyl)-1-piperazineacetic acid [[2-hydroxy-3-(2-propenyl)phenyl]methylene] hydrazide (PAC 1)	pro-apoptotic; activator of procaspase-3
Gambogic acid	apoptosis inducer; activates caspases and inhibits Bcl-2 family proteins

**Table 4.**  
*Caspase activators.*

strategy to kill cancer cells and can potentially help to reverse drug resistance. The most important cysteine protease in the caspase cascade is caspase 3 [50]. In a normal cell, capase-3 is typically in anactive form by an intramolecular electrostatic interaction favored by triplet of aspartic acid residues (also known as safety-catch). Although, there are indication that cell death can proceed in the absence of caspases, activation caspase family members is critical for execution of cell death in apoptotic cells. Several drugs for activating caspases exist (**Table 4**). An important caspase-inducing agent is apoptin, which is a proline-rich protein capable of inducing apoptosis in cancer cells in a selective manner. This protein is obtained from chicken anemia virus that causes tumor-specific apoptosis without interfering with normal cells. As such, apoptin is considered as a highly tumor-specific therapeutic agent [51, 52]. This drug is still in preclinical testing.

6. Conclusion

Due to its role in tissue homeostasis and cancer pathogenesis, apoptosis has received tremendous attention as a target for therapeutic intervention of cancer. Thus targeting apoptotic signaling networks is an important therapeutic strategy for cancer therapy. Many drugs have been developed that modulate different components of apoptotic signaling. However, many tumors ultimately develop

drug resistance against these drugs. Therefore there is a need to develop novel therapeutic agents and new treatment strategies that can reverse the drug-resistant phenotype of tumors and render these tumors sensitive towards therapeutic regimens. A limited number of FDA-approved drugs exist that directly target apoptotic pathways. Many of these drugs target the Bcl-2 family member such as Bcl-2 itself and Mcl-1. Caspase 3 activators are also being tested in preclinical settings. Other therapeutic strategies involve the use of drugs that activate the extrinsic pathway of apoptosis or tumor suppressor pathways or modulate tumor microenvironment. However, it is interesting to conclude that apoptosis can be induced in cancer cells by activating both the intrinsic and extrinsic pathways of apoptosis. However there is a need to identify therapeutic targets that are unique to cancer cells so that their modulation may not affect the normal surrounding cells. Thus, while targeting apoptotic signaling networks, there is a need to ensure tumor-specific apoptosis without inducing apoptosis in normal cells. We are hopeful that, in future, a better understanding of apoptotic signaling may lead to the development of novel anticancer drugs that could target different components of apoptotic signaling selectively and specifically. However, the lack of precise relationship between level of proteins involved in apoptosis and the clinical outcome poses a big challenge in the development of novel therapeutic agents and as such warrants in-depth understanding of apoptotic signaling networks.

## Acknowledgements

The first author is thankful to University Grants Commission, New Delhi, India for junior research fellowship (UGC-JRF). The corresponding author is grateful to SERB-National Postdoctoral Fellowship program and *Science and Engineering Research Board, Department of Science and Technology*, New Delhi, India (Grant Number: PDF/2016/002730) for financial support.

## Conflict of interest statement

The authors have declared that there are no conflicts of interest.

## Author Contributions

RAR and ST conceived the idea. ST wrote the chapter with the assistance from RAR, SKK and MB. All authors contributed to the chapter and approved the submitted version.

IntechOpen

### Author details

Sonia Thapa<sup>1,2</sup>, Rafiq A. Rather<sup>3\*</sup>, Shashank K. Singh<sup>1,2</sup> and Madhulika Bhagat<sup>3</sup>

1 Cancer Pharmacology Division, CSIR-Indian Institute of Integrative Medicine, Jammu, India

2 Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India

3 School of Biotechnology, University of Jammu, Jammu and Kashmir, India

\*Address all correspondence to: [rafiqueahmadd@gmail.com](mailto:rafiqueahmadd@gmail.com)

### IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 



## References

- [1] Wyllie, A.H., *Apoptosis: an overview*. British medical bulletin, 1997. **53**(3): p. 451-465.
- [2] Lowe, S.W. and A.W. Lin, *Apoptosis in cancer*. Carcinogenesis, 2000. **21**(3): p. 485-495.
- [3] Mattson, M.P., *Apoptosis in neurodegenerative disorders*. Nature reviews Molecular cell biology, 2000. **1**(2): p. 120-130.
- [4] Mattson, M.P., *Neuronal life-and-death signaling, apoptosis, and neurodegenerative disorders*. Antioxidants & redox signaling, 2006. **8**(11-12): p. 1997-2006.
- [5] Fernald, K. and M. Kurokawa, *Evading apoptosis in cancer*. Trends in cell biology, 2013. **23**(12): p. 620-633.
- [6] D'Arcy, M.S., *Cell death: a review of the major forms of apoptosis, necrosis and autophagy*. Cell biology international, 2019. **43**(6): p. 582-592.
- [7] Majtnerová, P. and T. Roušar, *An overview of apoptosis assays detecting DNA fragmentation*. Molecular biology reports, 2018. **45**(5): p. 1469-1478.
- [8] Jan, R., *Understanding apoptosis and apoptotic pathways targeted cancer therapeutics*. Advanced pharmaceutical bulletin, 2019. **9**(2): p. 205.
- [9] Wyllie, A., G. Beattie, and A. Hargreaves, *Chromatin changes in apoptosis*. The Histochemical Journal, 1981. **13**(4): p. 681-692.
- [10] Allen, R.T., W.J. Hunter III, and D.K. Agrawal, *Morphological and biochemical characterization and analysis of apoptosis*. Journal of pharmacological and toxicological methods, 1997. **37**(4): p. 215-228.
- [11] Chen, L., Y. Zeng, and S.-F. Zhou, *Role of apoptosis in cancer resistance to chemotherapy*. Current Understanding of Apoptosis-Programmed Cell Death, 2018.
- [12] Liu, B., et al., *Both intrinsic and extrinsic apoptotic pathways are involved in Toll-like receptor 4 (TLR4)-induced cell death in monocytic THP-1 cells*. Immunobiology, 2017. **222**(2): p. 198-205.
- [13] Mandal, P., A.L. McCormick, and E.S. Mocarski, *TNF Signaling Dictates Myeloid and Non-Myeloid Cell Crosstalk to Execute MCMV-Induced Extrinsic Apoptosis*. Viruses, 2020. **12**(11): p. 1221.
- [14] Salva, K.A., et al., *Epigenetically Enhanced PDT Induces Significantly Higher Levels of Multiple Extrinsic Pathway Apoptotic Factors than Standard PDT, Resulting in Greater Extrinsic and Overall Apoptosis of Cutaneous T-cell Lymphoma*. Photochemistry and photobiology, 2018. **94**(5): p. 1058-1065.
- [15] Gonçalves, I., et al., *sTRAIL-R2 (soluble TNF [tumor necrosis factor]-related apoptosis-inducing ligand receptor 2) a marker of plaque cell apoptosis and cardiovascular events*. Stroke, 2019. **50**(8): p. 1989-1996.
- [16] Campbell, K.J. and S.W. Tait, *Targeting BCL-2 regulated apoptosis in cancer*. Open biology, 2018. **8**(5): p. 180002.
- [17] Inoue-Yamauchi, A., et al., *Targeting the differential addiction to anti-apoptotic BCL-2 family for cancer therapy*. Nature communications, 2017. **8**(1): p. 1-14.
- [18] Chen, W.H., G.F. Luo, and X.Z. Zhang, *Recent advances in subcellular targeted cancer therapy based on functional materials*. Advanced Materials, 2019. **31**(3): p. 1802725.
- [19] Kalkavan, H. and D.R. Green, *MOMP, cell suicide as a BCL-2 family*

business. *Cell Death & Differentiation*, 2018. **25**(1): p. 46-55.

[20] Kale, J., E.J. Osterlund, and D.W. Andrews, *BCL-2 family proteins: changing partners in the dance towards death*. *Cell Death & Differentiation*, 2018. **25**(1): p. 65-80.

[21] Pihán, P., A. Carreras-Sureda, and C. Hetz, *BCL-2 family: integrating stress responses at the ER to control cell demise*. *Cell Death & Differentiation*, 2017. **24**(9): p. 1478-1487.

[22] Heckmann, B.L., B. Tummers, and D.R. Green, *Crashing the computer: apoptosis vs. necroptosis in neuroinflammation*. *Cell Death & Differentiation*, 2019. **26**(1): p. 41-52.

[23] Zhou, Z., et al., *Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells*. *Science*, 2020. **368**(6494).

[24] Velotti, F., et al., *Granzyme B in Inflammatory Diseases: Apoptosis, Inflammation, Extracellular Matrix Remodeling, Epithelial-to-Mesenchymal Transition and Fibrosis*. *Frontiers in immunology*, 2020. **11**: p. 2828.

[25] Chiusolo, V., et al., *Granzyme B enters the mitochondria in a Sam50-, Tim22-and mtHsp70-dependent manner to induce apoptosis*. *Cell Death & Differentiation*, 2017. **24**(4): p. 747-758.

[26] Wu, M., et al., *Phosphorylation of SET mediates apoptosis via P53 hyperactivation and NM23-H1 nuclear import*. *Neurobiology of aging*, 2018. **69**: p. 38-47.

[27] Mátyási, B., et al., *The function of NM23-H1/NME1 and its homologs in major processes linked to metastasis*. *Pathology & Oncology Research*, 2020. **26**(1): p. 49-61.

[28] Nosek, H., et al., *Tumor Necrosis Factor-Like Weak Inducer of Apoptosis*

*and Selected Cytokines—Potential Biomarkers in Children with Solitary Functioning Kidney*. *Journal of Clinical Medicine*, 2021. **10**(3): p. 497.

[29] Shermatov, K., et al., *Levels of Serum M30 and M65 Proteins as Biomarkers of Apoptosis in Children Exposed To Passive Smoking*. *Konuralp Tıp Dergisi*, 2018. **10**(3): p. 289-293.

[30] Burke, P.J., *Mitochondria, bioenergetics and apoptosis in cancer*. *Trends in cancer*, 2017. **3**(12): p. 857-870.

[31] Manchanda, A. and A. Arora, *Role of Apoptosis in Cancer Proliferation and in molecular target therapy*. *Baba Farid University Dental Journal*, 2019. **9**(2): p. 67-72.

[32] Shirjang, S., et al., *MicroRNAs in cancer cell death pathways: Apoptosis and necroptosis*. *Free Radical Biology and Medicine*, 2019. **139**: p. 1-15.

[33] Slattery, M.L., et al., *Dysregulated genes and miRNAs in the apoptosis pathway in colorectal cancer patients*. *Apoptosis*, 2018. **23**(3): p. 237-250.

[34] Zhang, G., et al., *Identification of cancer-related miRNA-lncRNA biomarkers using a basic miRNA-lncRNA network*. *PLoS One*, 2018. **13**(5): p. e0196681.

[35] Zhang, W., et al., *MiR-126 reverses drug resistance to TRAIL through inhibiting the expression of c-FLIP in cervical cancer*. *Gene*, 2017. **627**: p. 420-427.

[36] Rathore, R., et al., *Overcoming chemotherapy drug resistance by targeting inhibitors of apoptosis proteins (IAPs)*. *Apoptosis*, 2017. **22**(7): p. 898-919.

[37] Mohamed, M.S., et al., *Inhibitors of apoptosis: clinical implications in cancer*. *Apoptosis*, 2017. **22**(12): p. 1487-1509.

- [38] Trisciuglio, D. and D. Del Bufalo, *New insights into the roles of antiapoptotic members of the Bcl-2 family in melanoma progression and therapy*. Drug discovery today, 2021.
- [39] Suvarna, V., V. Singh, and M. Murahari, *Current overview on the clinical update of Bcl-2 anti-apoptotic inhibitors for cancer therapy*. European journal of pharmacology, 2019. **862**: p. 172655.
- [40] Kim, E.M., et al., *The p53/p21 complex regulates cancer cell invasion and apoptosis by targeting Bcl-2 family proteins*. Cancer research, 2017. **77**(11): p. 3092-3100.
- [41] Garner, T.P., et al., *Progress in targeting the BCL-2 family of proteins*. Current Opinion in chemical biology, 2017. **39**: p. 133-142.
- [42] Mattox, T.E., et al., *Novel RAS inhibitor, MCI-062, potently and selectively inhibits the growth of KRAS mutant pancreatic tumor cells by blocking GTP loading of RAS*. Cancer Res., 2019.
- [43] Cidado, J., et al., *AZD4573 is a highly selective CDK9 inhibitor that suppresses MCL-1 and induces apoptosis in hematologic cancer cells*. Clinical cancer research, 2020. **26**(4): p. 922-934.
- [44] Stewart, M.L., et al., *The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer*. Nature chemical biology, 2010. **6**(8): p. 595-601.
- [45] Luedtke, D.A., et al., *Inhibition of Mcl-1 enhances cell death induced by the Bcl-2-selective inhibitor ABT-199 in acute myeloid leukemia cells*. Signal transduction and targeted therapy, 2017. **2**(1): p. 1-9.
- [46] Hird, A.W. and A.E. Tron, *Recent advances in the development of Mcl-1 inhibitors for cancer therapy*. Pharmacology & therapeutics, 2019. **198**: p. 59-67.
- [47] Dubrez-Daloz, L., A. Dupoux, and J. Cartier, *IAPs: more than just inhibitors of apoptosis proteins*. Cell cycle, 2008. **7**(8): p. 1036-1046.
- [48] Holcik, M., H. Gibson, and R.G. Korneluk, *XIAP: apoptotic brake and promising therapeutic target*. Apoptosis, 2001. **6**(4): p. 253-261.
- [49] Schimmer, A., et al., *Targeting XIAP for the treatment of malignancy*. Cell Death & Differentiation, 2006. **13**(2): p. 179-188.
- [50] Fulda, S. and K.-M. Debatin, *Caspase activation in cancer therapy*, in *Madame Curie Bioscience Database [Internet]*. 2013, Landes Bioscience.
- [51] Maddika, S., et al., *Cancer-selective therapy of the future: apoptin and its mechanism of action*. Cancer biology & therapy, 2006. **5**(1): p. 10-19.
- [52] Los, M., et al., *Apoptin, a tumor-selective killer*. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 2009. **1793**(8): p. 1335-1342.